

GENETICS OF AN L_2 VENATION MUTANT IN *DROSOPHILA MELANOGASTER*

II. PATTERN EFFECTS

JAMES H. CARLSON

Department of Zoology and Entomology, The Ohio State University, Columbus, Ohio 43210^{1, 2}

ABSTRACT

Pattern effects of the mutant L_2 vein in *Drosophila melanogaster* were analyzed at 18°, 26° and 30°C. Pattern profiles were made by dividing the vein into 20 equal intervals. These intervals were classified as to the presence or absence of vein material. The most frequent interruptions were found in the basal region of the vein. The vein interval 3, lying between 10 to 15 percent of the distance from the proximal end, was found to be the most sensitive region. This interval showed the highest frequency of interruption (in more than 50 percent of the flies), regardless of the temperature variable. The distal region was much more stable.

Differential pattern responses were found associated with changes in temperature and in sex. A general pattern characteristic of the mutant stock was noted at all three experimental temperatures.

INTRODUCTION

The venation system of *Drosophila melanogaster* lends itself quite well to genetic studies. Accurate quantitative data can be obtained by measuring the amount of vein or crossvein associated with the many venation mutants in this organism that both decrease and increase the amount of venation present, relative to the wild type. Also, the embryology of *Drosophila* concerned with wing development has been extensively studied by various workers (Auerbach, 1936; Bodenstein, 1950; Chen, 1929; and Waddington, 1939, 1940). The general results of these embryological investigations can be summarized as follows. The prepupal venation system is not identical with that found in the adult. At the prepupal stage, there is a vein at both the anterior and posterior margins of the wing, and also one in the middle of the wing that divides distally into branches which will later become the third and fourth longitudinal veins. The precursor of the fifth longitudinal vein is also present. The wing then swells up, due to an accumulation of liquid, and resembles an inflated balloon. Elongated processes are formed at the bases of cells on the upper and lower surfaces of the wing and these are joined together. The veins appear at this time, the developmental sequence of which has been described by Waddington (1940).

In a previous paper (Carlson, 1966), the classification of the degree of interruption of the second longitudinal vein (L_2) in the mutant stock being evaluated was given by arbitrarily setting up six classes of interruption. These six classes were as follows:

- Class 0—Normal L_2
- Class 1—0–20% of L_2 absent
- Class 2—20–40% of L_2 absent
- Class 3—40–60% of L_2 absent
- Class 4—60–80% of L_2 absent
- Class 5—80–100% of L_2 absent

This system takes into account the total amount of absence of vein and is not designed to shed light upon the pattern of this absence (i.e. a fly with 15% interruption in the basal segment of L_2 , a fly with 15% interruption in the distal segment, a fly with 15% interruption in the central portion, and a fly with 5% basal, 5% central, and 5% terminal L_2 interruption are all classified alike in Class 1).

¹Present address: Department of Biology, Rider College; Trenton, New Jersey.

²Manuscript received February 14, 1969.

From previous studies (Carlson, 1960), it has been shown that temperature changes the expression and penetrance of this mutant. This paper reports on an attempt to analyze the effects of temperature on the pattern of L_2 interruption of this mutant system.

MATERIALS AND METHODS

The standard culturing medium of corn meal, agar, dextrose, yeast, and mold inhibitor was used to culture the flies. Cultures were incubated at $26 \pm 1^\circ\text{C}$, $30 \pm 1^\circ\text{C}$, and 18°C . At the latter temperature, variation was as high as at -4°C , this greater variation being due to a malfunctioning in the incubator detected, by means of a Taylor recording thermograph near the end of the experiment.

Matings at 26°C and 30°C involved single-pair replications, the parents of a given mating being placed initially in shell vials for 24 hours and then being transferred to half-pint bottles. Previous experience had shown that viability of the mutant stock was reduced at 18°C . Because of this reduced viability, four males and four females were used for matings at this temperature.

For analysis of pattern of mutant expression, the presence and absence of interruption was determined for the progeny of a given mating at each of twenty equally spaced intervals along the course of the second longitudinal vein. Wings from forty flies of each sex at each of the three experimental temperatures (18°C , 26°C , and 30°C) were dissected off and mounted on glass slides. The wings were projected from a Tri-Simplex projector. Entries of less than 80 in Table 1 are due to unusable wings (i.e. torn or distorted). A grid ruled into twenty-one equally spaced lines was superimposed upon the wing image so that the L_2 vein was divided into twenty equal segments. The presence or absence of vein in each 5% segment was scored. By changing the angle of the ruled grid so that the points of intersection of L_2 with L_3 , and of L_2 with the wing margin coincided with lines one and twenty-one of the grid, it was possible to compensate for variations in wing size.

Chi-square tests were performed by means of $k \times 2$ contingency tables, where 2 = interrupted vs. non-interrupted intervals, and k = males vs. females or temperature variables, depending upon the data used.

RESULTS

The most frequently found pattern of expression was an interruption of the basal segment of the L_2 vein. A more graphic picture of the pattern of expression found in the mutant stock can be obtained by determining the presence or absence of venation for chosen intervals along the length of the L_2 vein from the points of junction with L_3 and with the anterior margin of the wing. The values obtained from this method are given in Table 1.

TABLE 1

Results of interruption for wings of males and females raised at 18° , 26° and 30° C. for each of 20 equal L_2 intervals. The numbers under intervals denote how many veins out of the total exhibited presence of L_2

Sex	Temperature	← Basal INTERVALS Terminal →																				Total Wings
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
♀	18°	71	42	36	37	48	63	67	69	72	70	72	72	71	70	73	71	71	66	64	54	73
♂	18°	74	49	35	40	51	63	71	75	75	74	73	72	70	72	72	71	71	73	68	54	75
♀	26°	58	6	2	2	4	13	20	40	64	66	72	72	73	74	74	74	74	74	68		74
♂	26°	73	22	6	6	13	24	39	48	60	67	71	72	74	76	76	76	76	76	75	61	76
♀	30°	52	11	5	5	6	16	22	36	49	57	61	67	71	75	76	76	76	76	76	71	76
♂	30°	56	17	5	7	9	15	25	35	47	50	58	63	64	66	69	71	71	71	70	61	72

The population of left and right wings within a given sex and a single temperature show no significant differences for any given L₂ interval. For this reason, the values for left and right wings were combined in Figures 1, 2, 3, 4, and 5, and in Table 1. The plotting of these intervals yields a profile of the regional pattern of interruption characterizing the mutant stock (fig. 1, 2, 3, 4, 5).

If the vein-making ability of the mutant stock is dependent, in any given region of L₂, upon a variable concentration of some vein-forming substance (P) and a threshold (T) for the venation response, the frequency scale would not be an appropriate one on which to compare the effects of genetic and environmental variables affecting the mutant phenotype (Wright, 1926; House, 1953). Assuming normal distributions with equal variances of a substance P for each plotted interval of L₂, and a constant threshold for the venation effect, venation frequency for that interval can be thought of as that portion of the curve of variation exceeding the threshold. The relationship between the relative location of the mean and the threshold corresponding to any given frequency can also be thought of as that portion of the curve of variation exceeding the threshold. The relationship between the relative location of the mean and the threshold corresponding to any given frequency can be expressed as a normal deviate, $\frac{\bar{P}-T}{\sigma}$. With a frequency of 50 percent interruption, the mean and the threshold would be the same and $\frac{\bar{P}-T}{\sigma} = 0$. For venation frequencies greater than 50 percent, the normal deviates will be positive. Venation frequencies less than 50 percent will produce negative values. This change from the frequency scale to one measuring the concentration of P in units of standard deviation has been called the inverse probability transformation (Wright, 1926). The addition of five to the normal deviate value constitutes a probit and avoids the use of negative values.

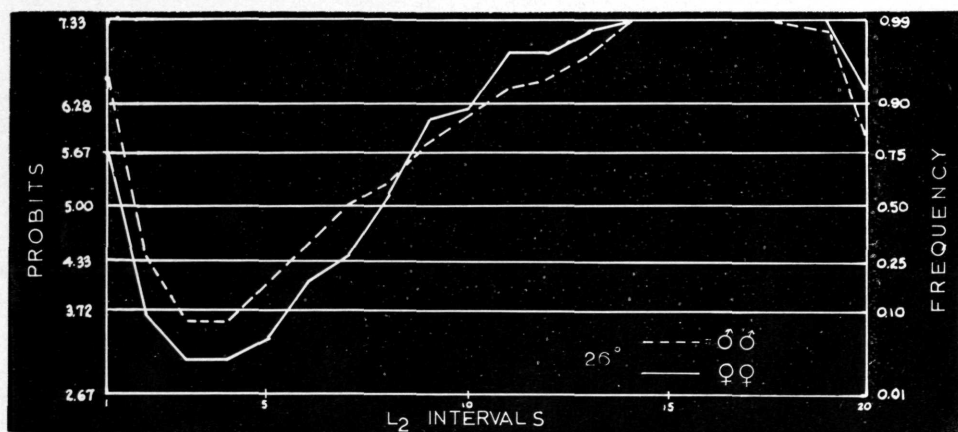


FIGURE 1. Pattern profiles of the mutant stock for males and females at 26°C.

The pattern profiles of males and females for 20 equal intervals of L₂ from base (interval 1) to tip (interval 20) are plotted in Figure 1. In this figure both probit (left) and frequency-of-vein-present (right) scales are given. The profiles indicate quite clearly the greater frequency of interruption of the proximal segments of L₂, as opposed to that in the more distal regions. Chi-square tests of the differences in frequency of venation between regions 3 vs 10, 3 vs. 15, and 3 vs. 20 all yield probability values less than 0.01.

An interesting feature of the difference in expression between males and females

is revealed by a comparison of the male and female profiles of Figure 1. Females show a significantly lower tendency for venation formation in intervals 1 and 2 (chi-square test, $p < .01$) than do males, yet in the regions 10–19 there is no significant difference in frequency of interruption between the sexes. In general, females are more likely to exhibit a slightly greater degree of interruption at the three temperatures used in this study.

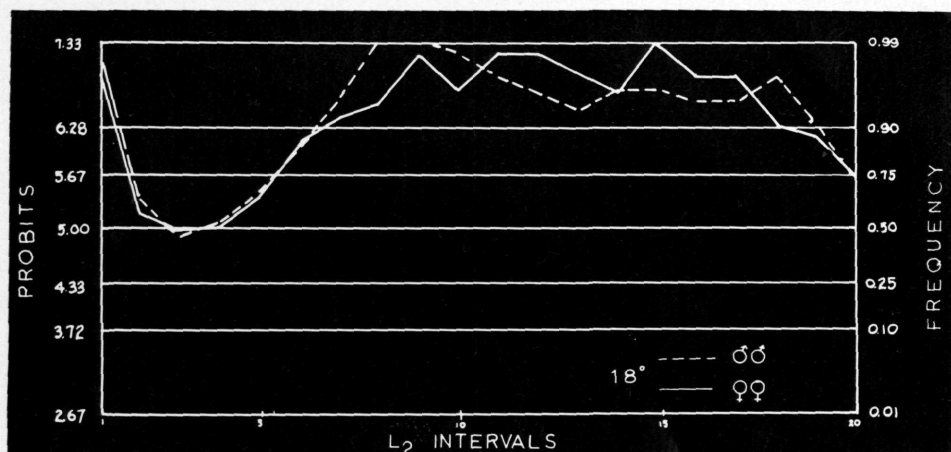


FIGURE 2. Pattern profiles of the mutant stock for males and females at 18°C.

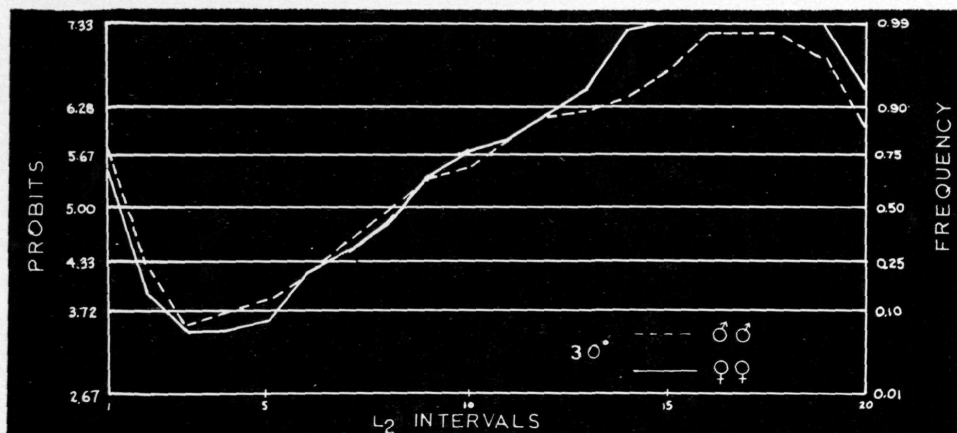


FIGURE 3. Pattern profiles of the mutant stock for males and females at 30°C.

For the purpose of evaluating the effects of temperature on pattern, profiles for males and females at 18°C and 30°C have been plotted in Figures 2 and 3 for comparison with those obtained at 26°C (fig. 1). In addition, the pattern profiles for males only and for females only at all three temperatures have been plotted in Figures 4 and 5, respectively.

Sex differences found at 26°C for intervals 1 and 2 were not found for these same intervals at 18° or 30°C (Table 1; fig. 1, 2, and 3). A common feature found at all three temperatures is that the profile curves for males and females

intersect in at least one point. At 30°C (fig. 3), the curves intersect at interval 9, and thereafter the frequency of L₂ vein absence in males is always greater than, or equal to, the frequency of L₂ vein absence in females, indicating a differential response for the various regions of the vein in the different sexes. There is, similarly, a reversal of temperature effect in the proximal region versus the distal regions of L₂, as seen in Figure 4 for 18° versus 26°C, and in Figure 5 for 18° versus

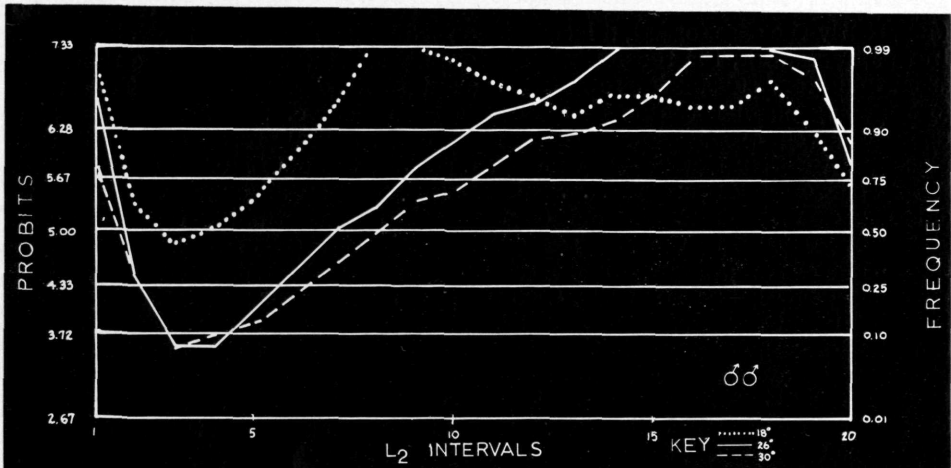


FIGURE 4. Pattern profiles of the mutant stock for males only at 18°, 26°, and 30°C.

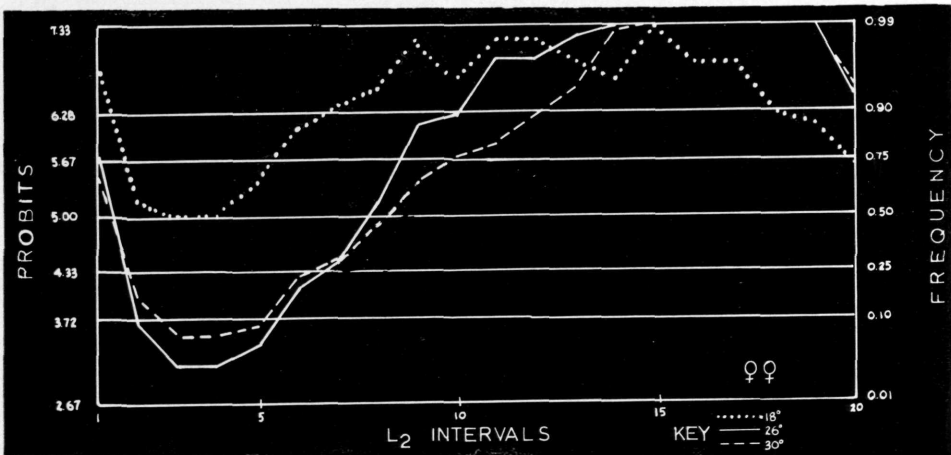


FIGURE 5. Pattern profiles of the mutant stock for females only at 18°, 26°, and 30°C.

30°C. Another common feature of pattern expression for all temperatures is the characteristic dip in number of wings with venation of the basal region, followed by a steady climb to a plateau of increased number of wings having venation in the central region, with a slight dip in number of wings showing venation in the two terminal intervals.

A chi-square analysis for differences in the frequency of venation in females at 18°C, 26°C, and 30°C for intervals 1, 2, 9, 10, and 20 yields significant differences

for these intervals at the 1% level. The same analysis for males yields significant differences (1% level) for intervals 1, 2, 9, and 10, but not for interval 20.

DISCUSSION

On the basis of Waddington's observations, House (1953) has postulated that the presence or absence of venation at any given point along the vein is a function of the presence or absence of basal processes sent out by the epithelial cells. In the absence of these processes, or following their disappearance after formation (apparently the case for L_2), a lacuna is formed which represents the vein channel. In this framework, the theoretical vein-forming substance P would be thought of as an inhibitory substance preventing the outgrowth of basal processes and therefore making possible the formation of the lacuna or vein. When P for a given region of a vein drops below a critical threshold, T, basal process outgrowth takes place and the vein lacunae are destroyed. Thus, to quote House, (1953, p. 323) "The vein-forming process . . . would appear to be an all or none reaction, the outcome of which is contingent on the conditions controlling the presence or absence of outgrowth of cytoplasmic processes in the cells over-and-under-lying the particular region in question."

This theoretical substance, P, then becomes identified as a material responsible for inhibiting basal-process outgrowth. Its supposed regional variation relative to a fixed threshold for inhibition forms the basis for the transformed frequency profiles of Figures 1, 2, 3, 4, and 5.

In connection with these profiles, note that changes in sex, genotype, and temperature do not affect the proximal and distal regions of L_2 in the same manner, even when the changes are measured on the probit scale. This differential response of proximal and distal regions of a longitudinal vein to changes in temperature and sex has also been demonstrated by House (1952; 1953) for the fourth longitudinal vein (L_4). In addition, changes in dosage of the cubitus interruptus (*ci*) allele show the same differential response of proximal and distal regions of L_4 as do temperature changes.

Perhaps a more interesting comparison is that involving the reversal of the effect of change in sex (fig. 1 and 3) and temperature (fig. 4 and 5) on the proximal versus distal region of L_2 found in the current study, and the reversal of the response of these regions of L_4 to changes in dosage of the *ci*⁺ allele in the presence of the engrailed (*en*) substitution found by House (1953). Whether this difference in behavior of the proximal and distal regions of the longitudinal veins in question results from some underlying basic difference in the development of venation in these regions or is peculiar to the mutants in question is unknown.

SUMMARY

General conclusions regarding pattern of L_2 interruption are as follows:

1. The most frequent interruption occurs at the proximal region of the vein. Interval 3 showed the highest frequency of interruption, being absent in more than 50 percent of the wings examined at all three temperatures.
2. The distal region of the vein was stable. At all temperatures, intervals 12 through 17 showed less than 10 percent average interruption.
3. Differential responses to temperature changes were found between sexes and between certain intervals.
4. A general pattern, characteristic of the mutant stock at all temperatures, was present.

ACKNOWLEDGEMENT

Appreciation is extended to Dr. Verl L. House of the Genetics Faculty, The Ohio State University, for his guidance of this research.

REFERENCES

- Auerbach, C.** 1936. The development of the legs, wings, and halteres in wild type and some mutant strains of *Drosophila melanogaster*. Trans. Roy. Soc. Edinburgh 58: 787-815.
- Bodenstein, D.** 1950. Biology of *Drosophila*, edited by M. Demerec. John Wiley and Sons, Inc., New York. 632 pp.
- Carlson, J. H.** 1960. A new mutant stock causing an interruption of the second longitudinal vein (L₂) in *D. melanogaster*. Drosophila Information Service 34: 74-75.
- . 1966. Genetics of an L₂ Venation Mutant in *Drosophila Melanogaster* I. Mode of Inheritance and Expression. Ohio Jr. Sci. 66: 340-346.
- Chen, T. Y.** 1929. On the development of the imaginal buds in normal and mutant *Drosophila melanogaster*. J. Morphol. 47: 135-199.
- House, V. L.** 1952. The interaction of some mutants affecting venation in *Drosophila malanogaster*. Genetics 37: 591-592.
- . 1953. The interaction of mutants affecting venation in *Drosophila melanogaster*. II. Additive and pattern effects of combinations of Hairless, engrailed, and cubitus interruptus. Genetics 38: 309-327.
- Waddington, C. H.** 1939. Preliminary notes on the development of the wings in normal and mutant strains of *Drosophila*. Proc. Nat. Acad. Sci. 25: 299-307.
- . 1940. The genetic control of wing development in *Drosophila*. Jr. Genetics 41: 75-139.
- Wright, S.** 1926. A frequency curve adapted to variation in percentage occurrence. J. Am. Stat. Assn. 21: 162-178.
-